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Study on the Cleaning of Tuberculous Cavity by Artificial Contamination

First Report. Experiments on Bacillus subtilis

By

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Introduction.

1. The significance of the contamination in tuberculous cavity.

It is true that the contamination of cavity exerts sometimes very bad influences upon pulmonary tuberculosis. But it is too much emphasized in general. On the contrary, as DR. SATA already proved (1899), the contamination accerelates remarkably the softening and the removing of caseous substances in tuberculous cavities and thus the inner walls of cavities can be easily cleaned. Therefore, if contaminated microorganisms are adequate, it may be possible that the contamination promotes the cleaning of tuberculous cavities without destruction.

On the other hand, when we examine the contents of tuberculous cavities in living bodies, contamination is surprisingly very rare. We assume that in living bodies tubercle bacilli may hinder the growth of other microorganism. This may be one reason of the difficulty of the cleaning of tuberculous cavities. We expect that the contamination with organisms, which are almost harmless to living individuals and can grow saprophitically in caseous substances and excrete antituberculous substances, will be convenient to the healing of tuberculous cavities.

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2. *Study on Bacillus subtilis group.*

As the first step we take up B. subtillis group. The reasons are as follows 1) Strains, which are capable of producing antituberculous substances, have been often detected within subtilis group, for examples Subtilin, Subtenolin and others. 2) It is assumed, that B. subtilis has no pathogenicity against human body. 3) As this group is very sensitive to penicillin or streptomycin, we can easily remove those bacilli supposing they exert prthogenicity to human body.

A. Fundamental experiments

Experiment 1. Selection of strains, which affect antibiotically to tubercle bacilli.

Method: Slide culture method was used in experiments of antagonism of B. subtilis and tubercle bacilli. Technical managements were following.

1) Slide glasses, which had been previously cut in half along their longitudinal axes and had been disinfected by dry heating, were put in an aseptic glass shell and were dried for 30 minutes in an incubator.

2) They were treated for 20 minutes in an bottle filled with 5% sulfuric acid solution of 37°C. (in order to remove contaminating bacilli in sputa.)

3) Sulfuric acid was washed away twice through two bottles containing sterile distilled water.

4) Then cultivation in ordinary test tubes. Culture media were KIRCHNER's media (10 % goat serum involved.)

5) After 24 hours cultivations, cultures were contaminated with a loopful of test bacilli.

6) After 6 days cultivation of tubercle bacilli slide glasses were taken out from culture media, and were washed with 10 % formalin solution. Thus culture were fixed and disinfected.

7) Dried in room temperature. Stained with Ziehl-Neelsen.

Microscopic examination. We compared these slides with control slides, which were taken out from culture media after 24 hours cultivation without adding contaminations, and select slides, in which the growth of tubercle bacilli were completely inhibited.

Results obtained: 202 strains of subtilis group were isolated and examined. 34 strains were capable of inhibiting tubercle bacilli by antagonistic cultivations.

Experiment 2. Selection of strains, which were not pathogeneous to animals but were able to live saprophitically in animal tissues.

Method: Male mice of about 15g body weights were inoculated intra-

peritoneally with 0,5 ml of bacillary saline suspensions, which involved 1/20 loopful of 24 hours cultivation of B. subtilis on nutrient agar. After 20 days animals were killed and bloods and peritoneal fluids were examined to detect living subtilis bacilli by culture methods. At the same time pathological changes in intraperitoneal organs were observed with naked eyes, and these organs were fixed and preserved in formalin solution.

Results obtained: Experiments were performed with 23 strains, which had been selected in the first experiment. 10 strains were selected. Animals, which were inoculated with these strains, increased their body weights until slaughter. From peritoneal fluids of these animals living bacilli were detected, but not from bloods. No pathological changes were recognized in intraperitoneal organs of these animals.

Adding experiments: After following third experiment we tested again on 5 selected strains in guinea pigs by the same methods as this chapter and confirmed that no pathogenicity were recognized.

Experiment 3. Selection of strains, which are sensitive to penicillin.

Method: One loopful of 4 days culture on nutrient agar were suspended in 2 ml of distilled water. One loopful of this suspension were inoculated into diluted broth, previously mixed with penicillin. Penicillin contents were 1.5, 0.5, 0.1, 0.01 and 0.005 units in 1.0 ml of media. After 18 hours and 5 days results were decided.

Results obtained: Aften 18 hours cultivations 5 among 10 strains, which had been selected in the second experiment, were inhibited their growth in 0.05 units penicillin. After 5 days cultivations every strain grew in 1.0 units penicillin solutions.

Experiment 4. Selection of strains, which are capable of growing in contents of tuberculous cavities.

Method: Each 1 ml of content of tuberculous cavity, which was collected aseptically from a patient and was not contaminated, was poured into disinfected small glass tubes. Add to each these glass tubes one droplet of highly diluted emulsion of test bacilli and stir to mix homogeniously. At once cultivate a loopful of this emulsion on a nutrient agar. Then glass tubes were incubated at 37°C. After 24 hours incubation one loopful of these emulsions was smeared on nutrient agar. It was decided from the numbers of colonies, whether test bacilli had increased in the contents of cavities after 24 hours incubations or not.

Results obtained: Until now we have examined 17 strains, which were selected by the first experiment. None of these strains could grow very good in the contents of cavities.

Discussion and summary: From fundamental experiments 1 ··· 3 we succeeded to select 5 strains of B. subtilis, which were assumed to be useful for the artificial contamination of tuberculous cavities. As for the forth experiment, it was performed just after following clinical experiment. We have learned that this forth experiment was very important as a fundamental experiment, because the results of this experiment ····· B. subtilis did not easily grow in the content of cavity ····· agreed very good with the results of clinical experiment ··· · B, subtilis, which had been inoculated int the cavities of patients, disappeared relatively early from the sputa of patients.

B. Clinicai experiments

Clinical experiments were performed with a strain S17, one of 5 strains previously selected in the fundamental experiments 1 ··· 3.

Method: In 4 patients experiments were performed. All these patients had very serious pathological changes in both lungs and cavities were giant (larger than 6 cm in diameter) in each patients. Therefore, every patient was too far advanced to be treated by collapstherapy. Sputum quantities were almost 50 ml a day. Tubercle bacilli in sputa were found as Gaffky $3 \sim 8$. Temperatures were normal in most cases, but in a very serious case temperature was inclined to be above 37°C, and sometimes temperature exceeded 38°C. 0.5 or 1.0 ml of saline suspensions of B. subtilis (5 mg or 10 mg wet weight of bacilli) were inoculated slowly into cavities by large needles.

Results obtained: Summary of results, obtained from 7 times inoculations in 4 patients were following.

1. Body temperature: In 3 cases temperature raised soon after inoculation with slight chill or without chill On the first or second day temperature reached to 38°C or more but without any treatment it became normal after one week. In another case no reaction of temperature was observed.

2. General conditions: Appetite decreased, when temperature raised, but it became normal soon after removal of fever. There were no other uncomfortable reactions than those, mentioned above.

3. Cough and sputum: There was no effect upon cough, except in one case, in whom cough became seldom by reason of reduction of viscosity of sputum.

4. Tubercle bacilli in sputum: In one case the number of tubercle bacilli increased temporarily and there were clumps of bacilli. Therefore, it seemed to be as if the cleaning of cavity wall had taken place, but this was a temporary phenomenon. In other cases there were no remakable changes.

5. B. subtilis in sputum: Although these bacilli were found in sputa 4 days after inoculations, generally they disappeared and could not be dete-

cted also in cultures already after 7 days.

Discussion and summary: As the clinical experiments began just early and treated cases were few and tested bacillus was only one strain, it may be too early to reach the conclusion. But as for only S17 strain it seems to be impossible to grow in human body. Therefore, there should be almost no possibility to acquire a good clinical result.

On the other hand pretty strong reactions occured in the treatments with bacilli as saprophitic as these subtilis bacilli, when their quantities were very large. Morover, good result may be expected when bacilli, which adapt easier to human body than those bacilli used above, are found. Therefore, we should be more careful in future experiments. We desire to continue the preceeding forth experiment using many other strains of subtilis group and to discover better strains. On the other hand, we will soon begin the fundamental experiment using microorganisms other than subtilis bacilli.